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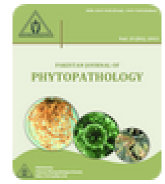

RESEARCH ARTICLE

Epidemiological Studies of Citrus Scab Disease in Different Growing Areas of District Sargodha

[a]Muhammad U. Shahzad, [a]Muhammad A. Zeshan, [a]Yasir Iftikhar, [b,c]Muhammad U. Ghani, [a]Sonum Bashir, [d]Salma Malik, [a]Komal Ambreen

[a] *Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha. Pakistan.*

[b] *Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad 38000, Pakistan.*

[c] *National-Regional Joint Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Inst. of Eco-Environmental and Soil Sciences, Guangdong Academy of Sciences, 808 Tianyuan Road, Tianhe District, Guangzhou, Guangdong 510650, China.*

[d] *Plant pathology Barani Agricultural Training Institute Rawalpindi. Pakistan.*

Corresponding Author:
Muhammad Ahmad Zeeshan, Email: muhammad.ahmad@uos.edu.pk

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A B S T R A C T

Citrus scab is a destructive disease that deteriorates the quality of the produce and makes it unfit for marketing and export. It also reduces fruit yield considerably. This study was planned to assess the role of epidemiological factors in the development of citrus scab disease in major citrus growing areas of Sargodha (Punjab-Pakistan). A prominent growing region (Silanwali Tehsil) was selected for survey of citrus orchards. Data on disease intensity irrigation sources and cultural practices was recorded with. The results indicated that orchards that were irrigated with canal water and that had weeds, dense canopies, and intercrops were highly infected with scab pathogens. There was a very strong relationship between disease incidence and severity; similarly, disease intensity increased with rise in temperature, relative humidity and wind speed. The study concludes that integrated management options with controlled irrigation and cultural practices are vital for sustainable, climate-resilient control of citrus scab.

Keywords: Citrus scab, Elsinoe, Qualitative, Export, losses.

INTRODUCTION

Citrus scab is an emerging fungal disease in citrus-producing regions worldwide. It is caused by *Elsinoë fawcettii* that affects many citrus cultivars such as grapefruit, mandarins, and sweet oranges (Jeffress *et al.,* 2020). Infected plants develop corky, raised pustules on fruit which reduce the market value of citrus fruits to a considerable level. Similar pustules on leaves and young stems decrease the frequency of photosynthetic activity and vitality of the plants leading to a lower yield in quantity and quality (Shin *et al.,* 2021).

The humid environment accelerates the growth of pathogen and it spreads through rain splashes, irrigation water, and wind. Fruits at early development stage are more vulnerable to infection by the pathogen which can persist within tissues for long time making it a serious challenge for the citrus growers (Pham *et al.,* 2025). Citrus scab disease has been established new citrus orchard settings very quickly due to climate change and easy global movement of the planting material. These factors have assisted the widespread distribution of

Elsinoë fawcettii spores and increased the susceptibility of early aged orchards, mainly planted with susceptible rootstocks under fluctuating environment (Chung, 2011). In the United States, scab disease was first detected on sweet oranges in 2010 in Texas which later on spread to other states California, Florida, and Arizona probably due to planting materials and weather conditions (Etebu and Nwauzoma, 2014). The pathogen continued to be spread rapidly in major citrus growing areas of US; in view of that U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), in cooperation with the California Department of Food and Agriculture (CDFA), expanded the quarantine zone by 32 square miles in January, 2025 (USDA APHIS, 2025).

Commonly, citrus scab detection is relied upon visual inspection followed by expert opinion; this could be a time-consuming method for disease detection and identification that lead to mistakes (Ghanei *et al.,* 2023; Iqbal *et al.,* 2024). Recently an AI based deep learning and hybrid meta-heuristic algorithms were used for citrus disease detection through which more than 99% accuracy was achieved in disease detection by using DenseNet-201 and AlexNet models. This method is durable and cost-effective for early disease identification, especially in citrus growing areas of Punjab, Pakistan, where it contributes almost 30% of total fruit production (Hassan *et al.,* 2025; Butt *et al.,* 2025). Although these smart tools are very efficient in disease detection, identification and diagnosis but these are not widely neither used yet nor easily accessible at common farmer's field. Moreover, the pathogen is developing resistance against the commonly used fungicides. Previously, epidemiological data was quantified more than a decade ago; however accurate disease assessment and management relies upon more recent and fresh data which is now prerequisite for effective disease management. There is a very scarce level of research on the integration of environmental factors with orchard level cultural practices for cost-effective disease management. Climate change and monoculture practices accelerate the pathogenic spread so the level of virulence under different cultural practices has not been widely studied before.

The objectives of the present study are to

- Assess the current incidence and severity of citrus scab across Sargodha's orchards
- Identify risk factors for disease outbreaks from microclimatic conditions, cultural practices and age of the orchard
- Integrated disease management of citrus scab disease

This study would be helpful in devising climate resilient and environment friendly disease management practices.

MATERIALS & METHODS

Experimental Details: A survey of citrus growing areas was planned in Silanwali Tehsil of District Sargodha (Punjab-Pakistan) to assess the disease intensity of citrus scab. Sargodha is best region for citrus especially Kinnow production, so all the survey was conducted in Kinnow orchards. The data was recorded repeatedly at fortnight intervals from the selected areas during multiple survey visits. There was stratified random selection of 7 Union Councils (UCs) from whole Tehsil followed by 3 Villages/UC; 2 orchards in each village one irrigated with tube well water and one with canal water. The orchards were selected by using stratified random sampling technique and data for scab disease incidence and severity was recorded. During selection of UCs, villages and orchards; it was ensured to include the maximum representing samples from whole of the population i.e. Tehsil. Apart from methods of irrigations, orchards were also observed for intercropping, cultural practices i.e. weeding and pruning and there effect on disease intensity was assessed. The orchards were selected from different age groups i.e. young or early planted, middle aged and older ones (more than 10 years of plantation).

Data recording: A standard protocol was followed for data recording; 5 citrus trees from each side of the orchard (EWNS) were selected and scab disease incidence data was recorded by using following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of symptomatic trees}}{\text{Total number of trees}} \times 100$$

After observing for symptoms from all sides of the orchards, the data was averaged and incidence percentage was calculated.

The data of disease severity was recorded by selecting 1 trees among the symptomatic and then narrowed down to branches, leaves and fruits from each side. The data was compiled afterwards and disease severity was calculated by using following formula:

$$\text{Disease severity (\%)} = \frac{\text{Number of symptomatic leaves or plant parts}}{\text{Total number of leaves or parts observed}} \times 100$$

The severity data was further categorized by using modified disease rating scale as devised by Ahmed *et al.,* (2020).

Disease rating scale for citrus scab severity

Grade	Scab disease severity (%)	Categories
0	No symptoms	HR/Immune
1	1-9	Resistant
2	10-19	Moderately resistant
3	20-29	Moderately susceptible
4	30-39	Susceptible
5	40-100	Highly susceptible

Data of environmental variables:

The data for weather variables was obtained from the NASA website (https://power.larc.nasa.gov/data-access-viewer/) by providing relative geographic coordinates and concerned dates.

STATISTICAL ANALYSIS

The data was subjected to descriptive analysis for an interpretable and summarized overview of the observations. The descriptive analysis was performed by using Microsft Excel, it provided information about data distribution i.e. central tendency and dispersion (the mean values for disease incidence and severity and how disease intensity varies across different sampling units). The strength of relationship between different variable i.e. severity and incidence; the effect of irrigation methods and cultural practices on disease intensity was assessed by correlation analyses (both Pearson and Spearman) by using SPSS software. The correlation between weather variables and disease intensity was also performed through SPSS. The prediction of disease severity on the basis of disease incidence and the effect of irrigation methods were analyzed through regression analyses which were performed by using SAS software.

Isolation of fungal pathogen (*Elsinoe fawcetti*): The symptomatic samples were collected from the surveyed fields and taken to the Plant Pathology Laboratory at College of Agriculture, University of Sargodha (Pakistan). The samples were washed with tap water to remove dust particles followed by washing in distilled water. The samples were kept for drying on blotter paper for almost 15 minutes and then surface sterilized with sodium hypochlorite followed by 3 washings of distilled water to remove the extraneous surface sterility to avoid from interruption with isolation process (Yaqoob *et al.,* 2024). The samples were again dried on blotter paper and then cut into small portions in such a way that a minute area of healthy tissue was also included as pathogen may move into that area. The small pieces were placed onto the PDA (potato dextrose agar) media which was already prepared by following the standard procedure. All the process of isolation was done under aseptic conditions in laminar flow chamber and plates were shifted to incubator till the appearance of fungal growth at 25 °C (Atiq *et al.,* 2024).

Morphological Identification and purification: After the appearance of fungal growth, a glass slide was prepared by following the standard procedure and subjected to microscopy for identification. The pathogen *Elsinoë fawcettii* was identified based on the already published characteristics both cultural and morphological. In pure culture colony color was near to black and microscopic identification was confirmed through conidiophores and conidia as described by Gopal *et al.* (2014). After confirmation, fungal culture was further multiplied for purification on to the PDA plates.

Pathogen confirmation through Pathogenicity Assay: After purification of the pathogen, petri dishes were flooded with sterile distilled water having 0.01% Tween-80 surfactant to harvest conidia. The suspension was passed through the sterile muslin cloth for removal mycelia. A hemocytometer was used to prepare the conidial suspension of 1×10^6 spores/mL).

Disease free and healthy citrus plants were sown in pots under screen house for the pathogencity assay. The plants were placed in controlled environment where light, humidity and temperature were maintained. The inoculation was done at 4-6 leaf stage and plants were observed for the appearance of symptoms.

The leaves of the test plants were punctured with sterile needle and droplet of the inoculum was inserted on to injured portion. The plants for comparison were treated with sterile distilled water. The inoculated plants were covered with transparent plastic bags and placed in a mist chamber for 2 days to maintain humidity as a favorable factor the fungal growth. The plants were continuously monitored for the appearance of symptoms and pathogen was re-isolated and confirmed through microscopy to fulfill Koch's postulates.

RESULTS

The effect of cultural practices was checked and evaluated on the orchards of Tehsil Silanwali regarding disease intensity (incidence and severity) Table. 1. The risk of disease onset was described as a result of multiple factors

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i.e. weeding, pruning and intercropping. Orchards having very high chances of disease outbreak depicted maximum disease incidence and severity and assigned highest disease rating scale category (4). These orchards were intercropped, abundant weeds and thick canopies.

Table 1. Scab intensity as affected by different cultural operations

Cultural Operation	Status	Disease incidence (%)	Severity (scale)
Weeding	Not Done	55.09	5
	Done	13.14	2
Intercropping	Present	56.75	5
	Absent	11.02	2
Pruning	Not Done	56.41	5
	Done	11.02	2

The compiled information of from whole Tehsil was of 7 UCs with 3 villages in each UC and 2 orchards from each village for comparison of irrigation water and its effect on disease intensity. The orchards irrigated with canal water are more susceptible for disease intensity of scab as compared to orchards received tube well irrigation (Table 2).

Table 2. Overall depiction of disease intensity as affected by irrigation water

Union Council	Village	Orchard	Irrigation	Scab incidence (%)	Scab severity
UC1	VA_1	O_1	Tube Well	34.07	2
UC1	VA_1	O_2	Canal	61.13	4
UC1	VA_2	O_3	Tube Well	39.01	3
UC1	VA_2	O_4	Canal	64.04	4
UC1	VA_3	O_5	Tube Well	31.12	2
UC1	VA_3	O_6	Canal	66.08	5
UC2	VB_1	O_7	Tube Well	28.14	1
UC2	VB_1	O_8	Canal	59.06	4
UC2	VB_2	O_9	Tube Well	33.08	2
UC2	VB_2	O_{10}	Canal	62.09	4
UC2	VB_3	O_{11}	Tube Well	36.14	2
UC2	VB_3	O_{12}	Canal	68.03	5
UC3	VC_1	O_{13}	Tube Well	30.11	2
UC3	VC_1	O_{14}	Canal	65.09	4
UC3	VC_2	O_{15}	Tube Well	27.02	1
UC3	VC_2	O_{16}	Canal	70.84	5
UC3	VC_3	O_{17}	Tube Well	35.57	2
UC3	VC_3	O_{18}	Canal	63.67	4
UC4	VD_1	O_{19}	Tube Well	32.05	2
UC4	VD_1	O_{20}	Canal	60.11	4
UC4	VD_2	O_{21}	Tube Well	29.13	2
UC4	VD_2	O_{22}	Canal	67.04	5
UC4	VD_3	O_{23}	Tube Well	31.12	2
UC4	VD_3	O_{24}	Canal	64.05	4
UC5	VE_1	O_{25}	Tube Well	26.15	1
UC5	VE_1	O_{26}	Canal	69.43	5
UC5	VE_2	O_{27}	Tube Well	30.07	2
UC5	VE_2	O_{28}	Canal	61.44	4
UC5	VE_3	O_{29}	Tube Well	33.05	2
UC5	VE_3	O_{30}	Canal	65.11	4

UC6	VF_1	O_{31}	Tube Well	29.12	2
UC6	VF_1	O_{32}	Canal	66.08	5
UC6	VF_2	O_{33}	Tube Well	34.44	2
UC6	VF_2	O_{34}	Canal	60.12	4
UC6	VF_3	O_{35}	Tube Well	28.13	1
UC6	VF_3	O_{36}	Canal	67.14	5
UC7	VG_1	O_{37}	Tube Well	31.11	2
UC7	VG_1	O_{38}	Canal	64.05	4
UC7	VG_2	O_{39}	Tube Well	27.14	1
UC7	VG_2	O_{40}	Canal	68.04	5
UC7	VG_3	O_{41}	Tube Well	30.26	2
UC7	VG_3	O_{42}	Canal	62.88	4

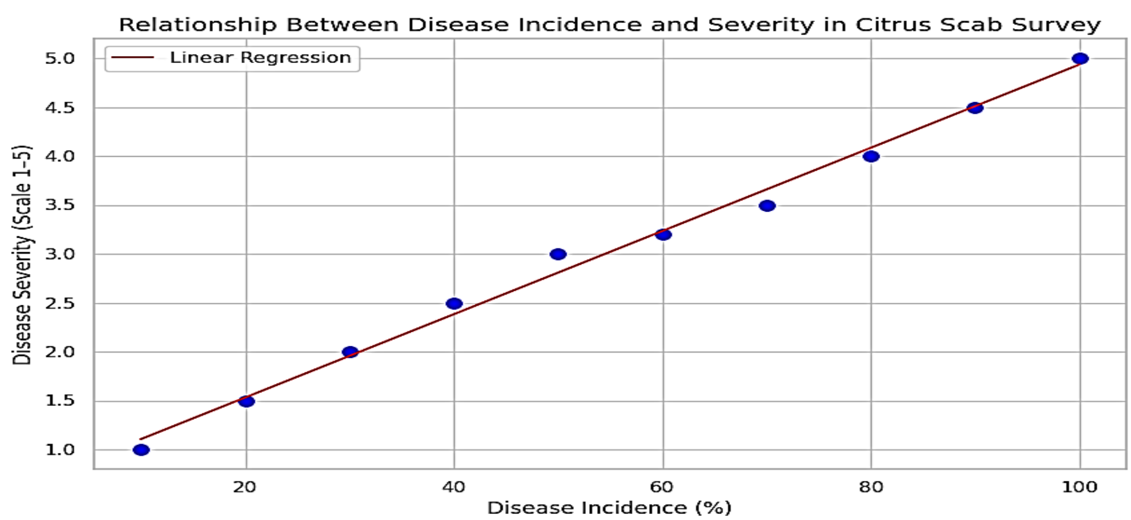

Figure 1. Relationship between disease incidence and severity

There is a clear illustration of mutual relationship between disease incidence and severity where fitted simple linear regression line is indicating the strong relation between both types of disease intensities (Figure 1). This is depicting a 94% strong relationship between severity and incidence. As the incidence increases severity also increases within individual plants as recorded during observations. There is a very obvious positive trend line on the observed points which is the indication of strong relation. The slope is describing that 1% increase in disease incidence; there would be 0.07 units increase in disease severity. The cluster of dots is very tight around the slope line indicating minimum residual error and maximum accuracy of prediction (Table 2). According to simple linear regression equation; there is a strong co-efficient of regression, mallow C(p) value is also ideal fit, high F value and less than 0.001 P value all are contributing to the fitness of the model.

Table 2. Simple linear regression equation for disease incidence and severity

Model Equation	R^2	C(p)	F-value	P-value
Severity = 0.07 × Incidence + 0.01	0.94	2.1	35.6	< 0.001

The model fitness was also confirmed by the trends of histogram distribution which was bell shaped symmetric and centered around zero indicating normally distributed errors (Figure 2).

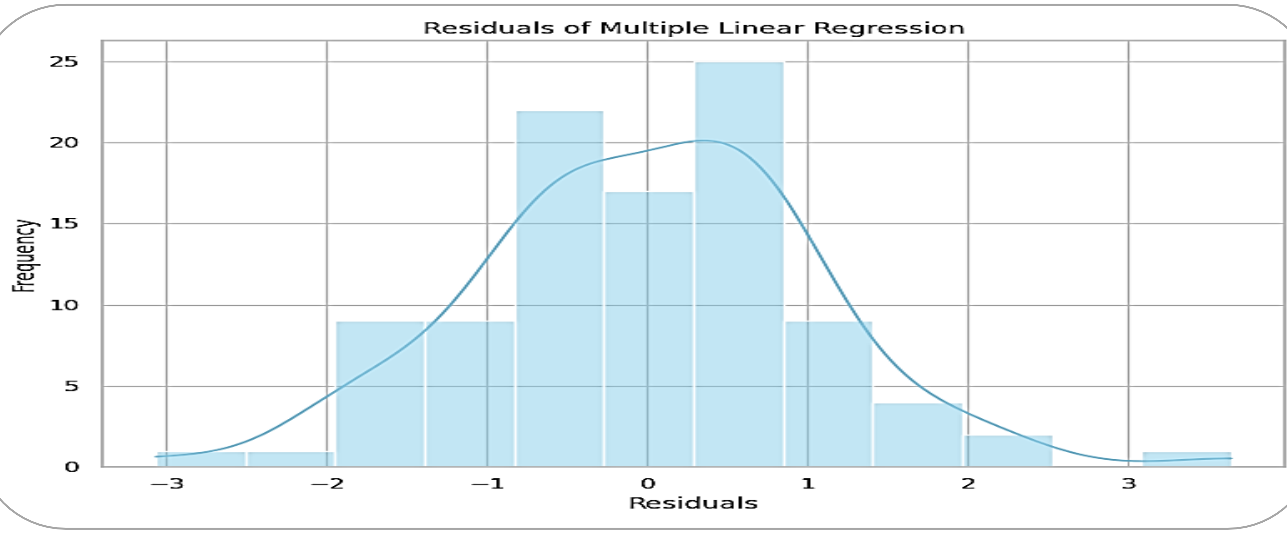

Figure 2. Histogram residuals for multiple linear regressions

Although irrigation method has a positive effect on disease intensity but it is not as stronger factor as others are. The variability for disease intensity in case of tube well is almost 28% which is less than as compared to canal irrigation (35%). Table 3. However, other model determining factors like Mallow's C(p), F-value, P-value and Co-efficient values are indicating strong effect of irrigation methods on disease intensity.

Table 3. Regression model for effect of irrigation methods on disease intensity

Irrigation Method	Co-efficient	R^2	C(p)	F-value	P-value
Tube Well	0.1999	0.285	1.98	6.39	< 0.001
Canal	0.3284	0.351	2.12	8.74	< 0.001

The effect of irrigation methods i.e. canal and tube well was well documented by using regression analysis. The dots are indicating the actual and predicted values of disease intensity as a result of irrigation methods. Dots closer to the dashed line are well describing the accuracy of the model; showing that canal water is more contributing towards disease intensity as compared to tube well irrigation (Figure 3).

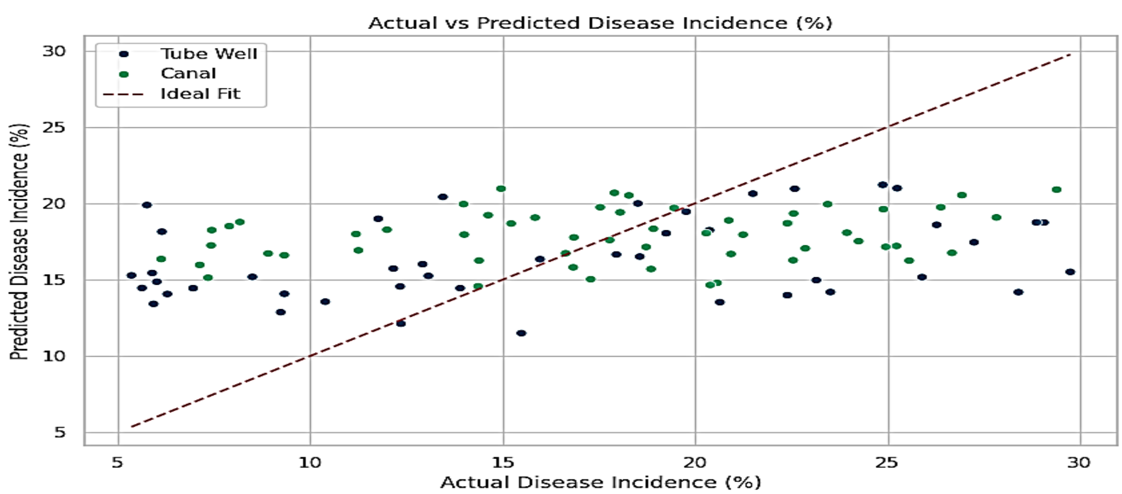

Figure 3. Predicted and actual disease incidence as affected by irrigation methods

The correlation analyses between disease incidence and severity conferred the same level of strength when evaluated both for Pearson and Spearman methods (Table 4). Correlation coefficient values for Pearson and

Spearman indicating the strong dependence of disease severity and incidence on each other. The lower P values in both methods are depiction of statistical significance of the data rather than a random chance. All the environmental variables affected the disease development in a positive manner. Disease intensity increased with increasing temperature, relative humidity, wind speed and rainfall.

Table 4. Correlation of environmental conditions with disease intensity of scab

Weather	Disease	Correlation (Pearson)	P-value	Correlation (Spearman)	P-value
Temperature (Max)	Incidence	0.98	0.0003	0.0000	1.00
Temperature (Max)	Severity	0.95	0.0003	0.0000	1.00
Temperature (Min)	Incidence	0.96	0.0003	0.0000	1.00
Temperature (Min)	Severity	0.94	0.0003	0.0000	1.00
Relative humidity	Incidence	0.97	0.0005	0.0000	1.00
Relative humidity	Severity	0.97	0.0005	0.0000	1.00
Wind speed	Incidence	0.95	0.0005	0.0000	1.00
Wind speed	Severity	0.95	0.0005	0.0000	1.00
Rainfall	Incidence	0.96	0.0003	0.0000	1.00
Rainfall	Severity	0.96	0.0003	0.0000	1.00

*All p-values < 0.05 indicate statistically significant correlations.

DISCUSSIONS

Overview of the study: The present experiment focused on the epidemiological factors affecting citrus scab disease development in core region of citrus (Kinnow) production i.e. Sargodha (Pakistan). The prominent factors considered for disease development were irrigation methods, cultural operations and weather variables. The incidence and severity of scab was high due to canal water irrigation, weeds, unpruned plants and intercrops. As multiple factors were deeply studied, it would be a valuable finding for the sustainable and integrated disease management option of citrus scab across Punjab (Pakistan).

Effect of irrigation methods: In present study, it was noted that there was higher disease incidence and severity in the orchards irrigated with canal water as compared to tube well irrigation. There was higher determination coefficient in case of canal water irrigation which depicted the strength of prediction if an orchard continuously receives canal irrigation. The range of disease incidence in case of canal water irrigation was (58-64%) while it was 27-34% in tube well irrigation. The reason behind more disease incidence due to canal water irrigation can be explored in such a way that it floods the field and enhances humid conditions and leaf wetness is prolonged which is the ideal conditions for *Elsinoe fawcettii* fungus for fruit and foliage penetration and infection (Pham *et al.,* 2025). Masheti *et al.,* (2024) reported that relative humidity increases the spore germination ability of the fungus and ultimately scab development. Chaudhari *et al.,* (2024) described that canal water produces more humidity in soil and microclimate of the plants as compared to drip irrigation. The more humid conditions in microclimate worsen the fruit development which is prone to be invaded by the insects and pathogens. The second most prominent reason for disease incidence in canal water irrigated orchards might be the untreated surface water that canals contains that may have fungal spores of different types increasing pathogenic loads in the field. Canal water covers more area as compared to tube well because it disperses the spores from infected to healthy plants. (Vishwakarma *et al.,* 2024).

Role of Weeding in disease development: Effect in microclimate of the plant: It is evident from the current study's findings that orchards where weeding was not performed showed more disease intensity. There was high disease incidence (55%) in the orchards where weeds were present as compared to orchards where weeding was done. *Elsinoe fawcettii* thrives best under humid conditions; weeds if present on the soil stores more water and increase the humidity in microclimate of the plant that creates favorable environment for the fungus. These findings were also confirmed by the earlier researchers; according to Li *et al.,* (2023), dense weed cover considerably enhanced soil humidity and microbial activity that ultimately nourish pathogen survival and sporulation.

Effect on pathogen survival and spread: Weeds apart from conserving the moisture serve as a prominent source of pathogen survival as an alternate host and continuity of the infection cycles thus supporting in disease development events. Weeds also hinder the cultural operations of pruning, spray efficiency and sanitation to make the field unsuitable for pathogen survival (Parajuli *et al.,* 2025). Weeds are further a source of attraction for insects and rodents that can spread spores of fungi from the infected plants to healthy ones. The insects and rodents can also injure the plants making them prone for pathogen attack and infection (Demis, 2024).

Effect of pruning on disease development: The orchards where pruning was not done showed more disease incidence (56%) and severity (5 rating scale) in current survey. The percentage of disease incidence was reduced to 11% and rating scale of severity was declined to number 2 in case of pruned orchards. Dense canopies favor the fungal growth on fruits and foliage by the conservation of moisture due to poor ventilation. In pruned orchards there were more air circulation and less moisture conservation making the growing conditions unfavorable for the *Elsinoe fawcettii* spores (Yang *et al.,* 2025). Further, pruning is necessary to remove the source of preservation for the pathogen spores in the microclimate of the citrus plants. Infected branches and twigs are the primary reservoir for the pathogen inoculum which continues the life cycle of pathogen (Matias *et al.,* 2023).

Pruning not only makes the orchard less feasible for pathogen growth but also physically removes it from premises of the orchard. This is the most cost-effective and eco-friendly management strategy for citrus scab.

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Contribution of Authors:

Muhammad U. Shahzad	:	Conceptualization
Muhammad A. Zeshan	:	Supervision of the project
Yasir Iftikhar	:	Formal analysis
Muhammad U. Ghani	:	Software
Sonum Bashir	:	Methodology
Salma Malik	:	Investigation
Komal Ambreen	:	Data curation & validation